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Robert Graham

A note on the presence of *B. Botulinus*
Type A in the excretions of poliomyelitis
patients.

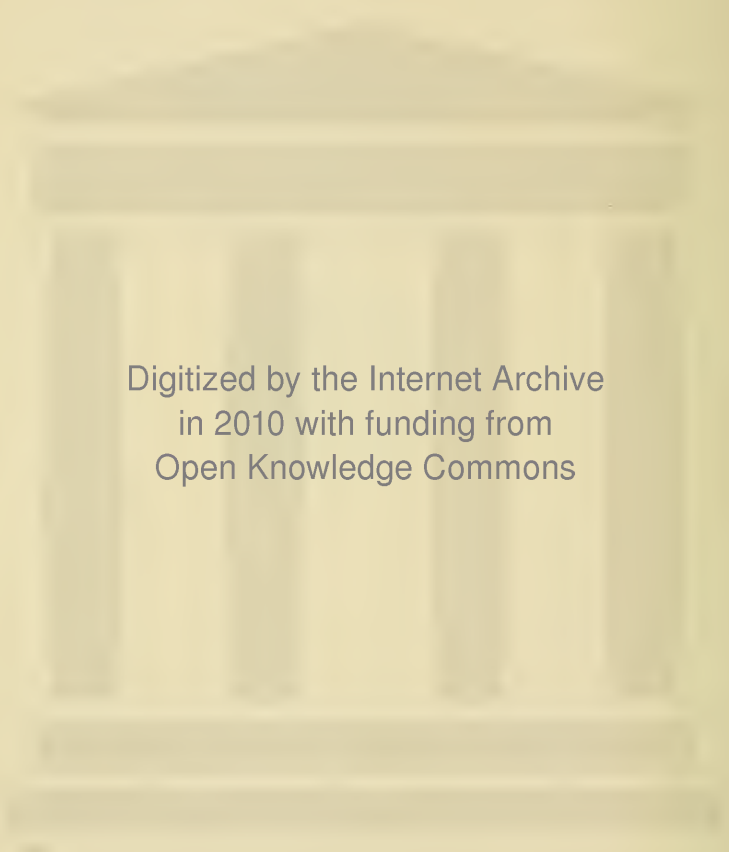
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A NOTE ON THE PRESENCE OF B. BOTULINUS TYPE A IN THE EXCRETIONS OF POLIOMYELITIS PATIENTS

By ROBERT GRAHAM AND EDGAR BARGER
LABORATORY OF ANIMAL PATHOLOGY AND HYGIENE



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URBANA

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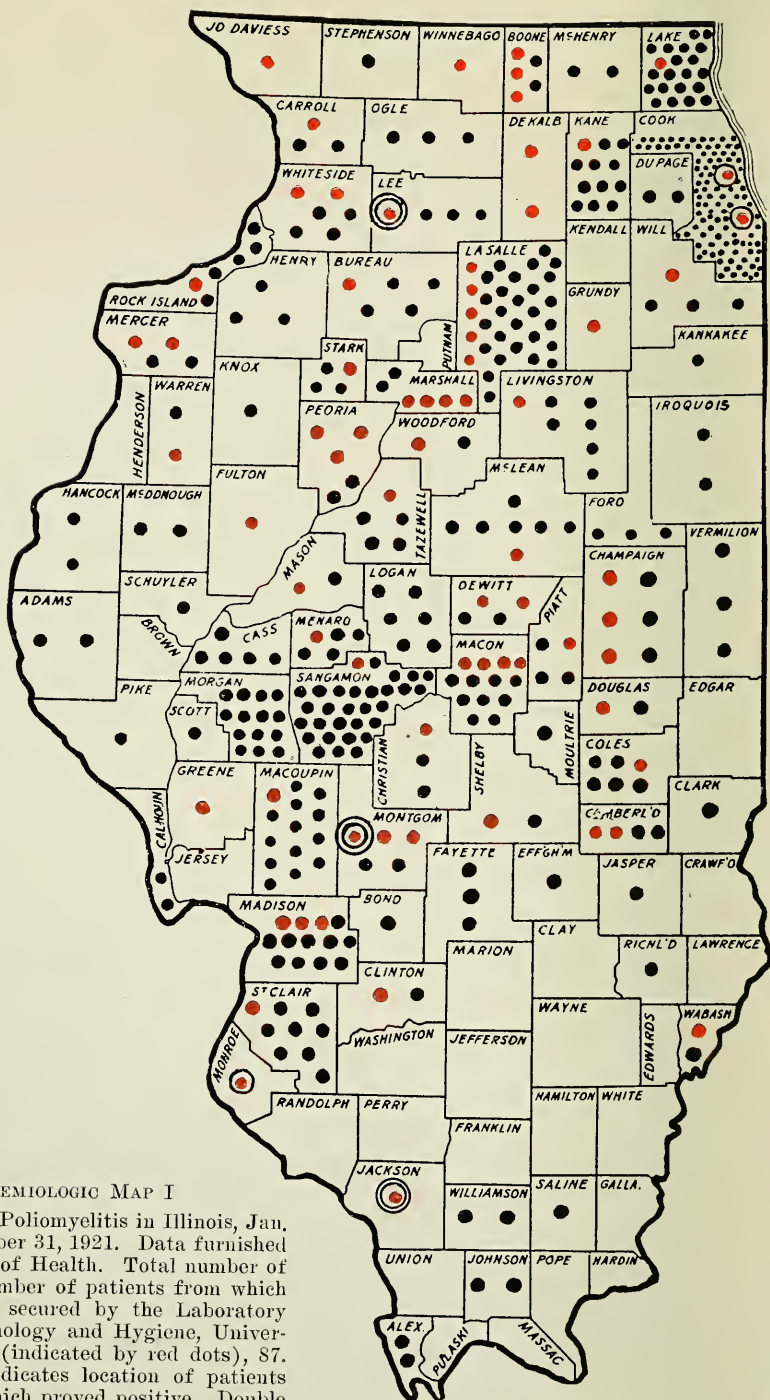
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EPIDEMIOLOGIC MAP I

Outbreaks of Poliomyelitis in Illinois, Jan. 1, 1920, to October 31, 1921. Data furnished by State Board of Health. Total number of cases, 463. Number of patients from which specimens were secured by the Laboratory of Animal Pathology and Hygiene, University of Illinois (indicated by red dots), 87. Single circle indicates location of patients excretions of which proved positive. Double circle indicates location of patients excretions of which proved positive but which are not discussed in this leaflet.

A NOTE ON THE PRESENCE OF B. BOTULINUS TYPE A IN THE EXCRETIONS OF POLIOMYELITIS PATIENTS

BY ROBERT GRAHAM AND EDGAR BARGER,
LABORATORY OF ANIMAL PATHOLOGY AND HYGIENE, UNIVERSITY OF ILLINOIS

Thru the cooperation of health officials, hospitals, and practicing physicians of Illinois,¹ specimens of excretions from children suffering from poliomyelitis have been submitted to the Laboratory of Animal Pathology and Hygiene for examination. Sterile containers were mailed to the attending physician and the samples of urine and feces as collected were cultured immediately upon arrival at the laboratory. The purpose of this leaflet is to record the presence of B. botulinus type A in one feces and two urines of children during the convalescent period of a poliomyelitis infection.² The bacteriologic studies here reported are limited to samples of urine and feces from cases diagnosed as poliomyelitis.

The possibility of B. botulinus as a saprophytic contaminant of the alimentary tract of healthy children as well as the possibility of this microorganism being present in the normal genito-urinary tract is not disregarded in the preliminary investigations. Furthermore, the method employed in collecting specimens may not have sufficed to eliminate extraneous contamination. In any event, it seems a noteworthy fact that B. botulinus should be encountered in excretions from the human body, and even tho upon further study it should be established that this microorganism bears no relation to the neurotropic virus of poliomyelitis or an analogous symptom complex in children, the widespread character of B. botulinus in nature will be more fully understood.

Poliomyelitis as an investigational problem came to our attention in connection with studies conducted during the past five years relative to the etiologic character of certain obscure toxemic syndromes in domestic animals. The results of these studies have shown that

¹ Grateful acknowledgment is hereby made for the helpful cooperation and assistance given by the Illinois State and Chicago Boards of Health, as well as the Cook County and Durand Hospitals of Chicago, Dr. C. W. East, Dr. J. J. McShane, Dr. C. G. Appelle, Dr. W. E. Schowengerdt, Dr. Butler, and many other practitioners of human medicine.

² Since this report was written, three specimens of urine (188, 242, and 395) from three other convalescent cases of poliomyelitis have upon bacteriologic examination revealed the presence of B. botulinus type A. The geographic locations of these three cases are indicated on epidemiologic Map I by a red dot surrounded by two circles.

botulism is a rather common disease thruout the Mississippi valley, affecting swine, chickens, cattle, sheep, and horses. (See Maps III and IV.) Notwithstanding the fact that Flexner and others working in the field of poliomyelitis have disregarded the possible relation between spinal paralysis in farm animals and poliomyelitis in children, certain clinical and epidemiologic observations in animal botulism with special reference to poliomyelitis in children as it occurred in Illinois during the late summer months of 1921 failed to clearly and decisively eliminate the possible etiologic connection between all cases of poliomyelitis and botulism in animals.

A knowledge of the varying symptoms of botulism in animals suggested that the clinical aspects of a botulinus intoxication in man might also vary markedly.¹ The clinical analogy between spontaneous botulism in nursing lambs as it occurred in Champaign county, Illinois (1917), to poliomyelitis in children also suggested the possibility of a common etiologic factor. Furthermore, the resistance of the bovine species to contaminated rations pointed to the possible danger of milk from apparently healthy lactating animals consuming contaminated grains and hays. Experimental data bearing on the relation of *B. botulinus* and toxin in the feed of the healthy lactating animal to the presence of the virus in fresh milk apparently suggest an explanation of the sporadic occurrence of some cases of spontaneous botulinus intoxication in the nursing young animals. The specific toxic character of milk from apparently healthy animals ingesting definite amounts of botulinus virus can be occasionally demonstrated in guinea pigs. In one instance Dingman² rather definitely recorded the possible relation of the milk supply to the occurrence of a disease in children diagnosed as poliomyelitis (New York, 1916). In three widely separated boarding houses patronizing the same milk dairy, cases of poliomyelitis were observed. In three other families in the community which were furnished milk from the same supply no cases of poliomyelitis occurred. An investigation revealed the fact that the milk was always boiled by the latter three families while the former three families used the milk raw. If Dingman was dealing with cases of botulism, other possible modes of transmission are obviously not excluded in view of the fact that botulinus toxin and spores have been demonstrated in other foods as well as in the larvae of the *Lucilia caesar* and in the mature fly.

¹ Recently, this contention was confirmed in connection with an acute gastrointestinal disturbance in children from eating fig newtons in one family and bread in another which upon examination were found to contain *B. botulinus* type A. The symptoms were not analogous to known cases of botulism in adult persons.

² Dingman, J. C. New York State Journal of Medicine, 16, 589. 1916.

The possible significance of the experimental findings in the milk of lactating animals consuming contaminated rations, if any, in relation to the health of the milk-consuming public suggested the occurrence of some disease of a cryptogenic etiology in man. The conjecture that such a disease might more often occur in children than in adults would naturally follow because of the dietary difference or varying tolerance of the two groups. A resumé of the literature on prevailing paralytic diseases of children with particular reference to numerous reports on poliomyelitis and the modes of transmission presents a conception of poliomyelitis which conflicts with the disease as experimentally produced and fails to harmonize with the generally accepted ideas regarding other infectious diseases. In this connection the possibility of a food transmission is not entirely disregarded. These and other facts suggested the advisability of attempting a comparative study of poliomyelitis in children from the standpoint of pathogenic anaerobes, with particular reference to *B. botulinus*.

Observations in animal botulism indicate that the symptomatology of a botulinus intoxication in children might not be easily recognized on the basis of the syndrome of this intoxication observed in mature persons. In fact, there has been no more perplexing problem in animal botulism than the clinical diagnosis of the disease, not only in different animal species, but in the same specie of different ages. If this phenomenon is subject to interpretation it may be explained on the basis that the toxin may affect or localize in different nerve centers and thus induce various forms of local paralysis. In view of the fact that a specific botulinus intoxication in animals has been clinically confused with acute bacterial infections, rabies, and deficiency diseases, it is even possible to suggest that the acute and more easily diagnosed cases of botulism in domestic animals and man constitute but one type of the existing, yet unrecognized, forms of this disease. The syndrome encountered in the classical picture of poliomyelitis as observed in different patients, however, suggested to the writers the possible presence of a neurotropic virus resembling the action of botulinus toxin.

A CLINICAL AND EPIDEMIOLOGICAL NOTE OF THE POLIOMYELITIS PATIENTS

Three of the cases of poliomyelitis in children, wherein *B. botulinus* type A was encountered in the excretions, were reported on the following epidemiologic cards of the Illinois State Department of Health:



FIG. 1.—PATIENT 154

PATIENT 154

ILLINOIS STATE DEPARTMENT OF HEALTH										CASE NO.
COUNTY										OUTBREAK IN FAMILY
CITY OR TOWNSHIP										
NAME										POLIOMYELITIS
DATE Sept. 12, 1921.										
AGE 4	SEX F	MARITAL STATE Single		COLOR W.	NATIONALITY Am.		OCCUPATION			
Years		Male—Female		Married—Single—Widowed		White—Black		(Of Mother, if minor)		
ADDRESS: STREET		NUMBER		FLOOR 1		ROOM NO.		CARE OF		
REPORTED BY Dr. Zoda D. Lumley										DATE Sept. 13, 1921.
THE HOUSE: GENERAL CONDITION Fair										NUMBER OF FAMILIES 1
STABLE NEAR? (YES) (NO) Yes										EXPOSED GARBAGE, ETC., NOTED? (YES) (NO) Yes
THE HOME: GENERAL CONDITION (GOOD) (BAD) Fair										FLIES (YES) (NO) Yes
										VERMIN (YES) (NO) No
THE FAMILY: ADULTS 3		CHILDREN 2		1		ON PREMISES SINCE 1 month				
		0—5 years		6—10 years		11—20 years				
EXPOSURE TO INFECTION: ANY CONTACT, DIRECT OR INDIRECT, WITH OTHER CASE? (YES) (NO)										
DETAILS, DATES OF CONTACT										
INSPECTOR'S DIAGNOSIS Acute anterior poliomyelitis										ONSET DATE Sept. 12th
PARALYSIS: WHEN FIRST APPEARED Sept. 13,										PARTS AFFECTED left arm
GENERAL SYMPTOMS: RESPIRATORY No										GASTRO INTESTINAL No
TYPE: 1 ABORTIVE										2 SPINAL Yes
										3 MENINGEAL
										4 CEREBRAL
										LUMBAR PUNCTURE DATE No
										RESULT
PRECAUTIONS OBSERVED: ISOLATED (YES) (NO) Yes										DEATH No
										FROM
FOOD HANDLERS IN FAMILY (YES) (NO) REPORT No										CHILDREN ATTEND SCHOOL AT
										EXCLUDED Yes
REMOVAL AND RENOVATION ORDERED Yes-screens										ASSIGNED TO Dr. Henry Reis, Belleville
										RETURNED

(16150-10M-3-14)

Anamnesis

Sept. 6—Child indisposed. Vomiting and pains in epigastrium.

Sept. 10, 11—Slight headache.

Sept. 12—Headache, backache, pain in right arm. Sensation in left arm, described as "worms crawling inside of arm."

Sept. 13—Left arm showed paralysis of long flexors and shoulder muscles. Slight constipation relieved by mild cathartic.

Diagnosis: Poliomyelitis

Urine as submitted in sterile container September 20, 1921, proved positive to B. botulinus type A.



FIG. 2.—PATIENT 269

PATIENT 269

ILLINOIS STATE DEPARTMENT OF HEALTH					CASE NO.	
COUNTY					OUTBREAK IN FAMILY	
CITY OR TOWNSHIP					POLIOMYELITIS	
NAME					DATE <u>Sept. 14, 1921</u>	
AGE <u>20 mo</u>	SEX <u>F</u>	MARITAL STATE <u>S</u>	COLOR <u>W</u>	NATIONALITY <u>U.S.</u>	OCCUPATION	
<small>Years</small>	<small>Male—female</small>	<small>Married—Single—Widowed</small>	<small>White—Black</small>	<small>(Of Native of minor)</small>		
ADDRESS: STREET <u>Western Ave.</u>		NUMBER <u>181</u>	FLOOR <u>2</u>	ROOM NO. <u>6</u>	CARE OF <u>Dr. Butler</u>	
REPORTED BY <u>H.O.</u>		ADDRESS		DATE		
THE HOUSE:		GENERAL CONDITION <u>Fair</u>		NUMBER OF FAMILIES <u>1</u>		
STABLE NEAR? (YES) (NO) <u>no</u>		EXPOSED GARBAGE, ETC., NOTED? (YES) (NO) <u>no</u>				
THE HOME: GENERAL CONDITION (GOOD) (BAD) <u>P</u>		FLIES (YES) (NO) <u>no</u>		VERMIN (YES) (NO) <u>no</u>		
THE FAMILY: ADULTS <u>2</u>		CHILDREN <u>1</u>		ON PREMISES SINCE <u>3 years</u>		
<small>0-4 years</small>		<small>5-10 years</small>		<small>11-20 years</small>		
EXPOSURE TO INFECTION; ANY CONTACT, DIRECT OR INDIRECT, WITH OTHER CASE? (YES) (NO) <u>no</u>						
DETAILS, DATES OF CONTACT						
INSPECTOR'S DIAGNOSIS <u>Acute anterior poliomyelitis</u>					ONSET: DATE <u>9-3-21</u>	
PARALYSIS: WHEN FIRST APPEARED <u>9-9-21</u>		PARTS AFFECTED <u>Right & left, upper & lower extremities</u>				
GENERAL SYMPTOMS: RESPIRATORY <u>yes</u>		GASTRO INTESTINAL <u>yes</u>				
TYPE: 1 ABORTIVE	2 SPINAL <u>yes</u>	3 MENINGEAL	4 CEREBRAL	LUMBAR PUNCTURE: DATE		RESULT
PRECAUTIONS OBSERVED: ISOLATED (YES) (NO) <u>yes</u>		DEATH		FROM		
FOOD HANDLERS IN FAMILY (YES) (NO) REPORT <u>no</u>		CHILDREN ATTEND SCHOOL AT <u>no</u>		EXCLUDED		
REMOVAL AND RENOVATION ORDERED		ASSIGNED TO <u>S.S. Winner, M.D.</u>		RETURNED		

(10199-10M-8-18)

Anamnesis

- July — Intermittent attacks of diarrhea and vomiting.
- Sept. 3—Child fell down stairs. Severe cold with purulent green nasal discharge. Diarrhea had subsided during the two weeks preceding this date.
- Sept. 5—Diarrhea, fever, attitude dull. Malaria suspected.
- Sept. 6—Fetid diarrhea, containing mucus. Spasmodic twitching of facial muscles, neck stiff; no Brudzinski, negative Babinski, respirations regular, lungs normal, pulse 140, temperature 102.2°. Glands in neck slightly enlarged.
- Sept. 9—Patient extremely toxic, stares at ceiling, irrational at times, abdominal breathing only, foot pendulent, slight opisthotonos, temperature 102°. Reluctant about moving lower extremities.
- Sept. 10—Paralysis of upper and lower extremities.

Diagnosis: Poliomyelitis

Stool as submitted in sterile container September 26, 1921, proved positive to *B. botulinus* type A.



FIG. 3.—PATIENT 276

PATIENT 276

ILLINOIS STATE DEPARTMENT OF HEALTH										CASE NO.		
COUNTY										OUTBREAK IN FAMILY		
CITY OR TOWNSHIP										POLIOMYELITIS		
NAME										DATE		
AGE	5	SEX	M	MARITAL STATE	S	COLOR	W	NATIONALITY	American	OCCUPATION		
Years		Male	Female	Married	Single	Widowed	White	Black	(If Street, H. Phone)			
ADDRESS STREET	Division			NUMBER	1737	FLOOR	4th	ROOM NO.		CARE OF	Dr. Levin	
REPORTED BY	Dr. L. M. Levin					ADDRESS				DATE		
THE HOUSE:	GENERAL CONDITION					Poor			NUMBER OF FAMILIES			about 10
STABLE NEAR?	(YES) (NO)	no			EXPOSED GARBAGE, ETC., NOTED?	(YES) (NO)	Yes					
THE HOME: GENERAL CONDITION (GOOD) (BAD)	F			FILIES (YES) (NO)	yes			VERMIN (YES) (NO)	Yes			
THE FAMILY: ADULTS	2			CHILDREN	1			ON PREMISES SINCE			2 months	
0-3 years 6-10 years 11-20 years												
EXPOSURE TO INFECTION: ANY CONTACT, DIRECT OR INDIRECT, WITH OTHER CASE?	(YES) (NO)	no										
DETAILS, DATES OF CONTACT												
INSPECTOR'S DIAGNOSIS	acute anterior poliomyelitis							ONSET DATE	9/16/21			
PARALYSIS: WHEN FIRST APPEARED	9/19/21			PARTS AFFECTED	Lower left extremity							
GENERAL SYMPTOMS: RESPIRATORY												
GASTRO INTESTINAL												
TYPE: 1 ABORTIVE	2 SPINAL			3 CERVICAL	4 CEREBRAL			LUMBAR PUNCTURE, DATE	100 cell			
Hospitalized												
increased pressure												
PRECAUTIONS OBSERVED, ISOLATED (YES) (NO)	Screens			DEATH	FROM			cloudy				
FOOD HANDLERS IN FAMILY (YES) (NO) REPORT	No			CHILDREN ATTEND SCHOOL AT	EXCLUDED							
REMOVAL AND RENOVATION ORDERED	ASSIGNED TO			S. S. Winner M. D.			RETURNED					

(10100-10M-8-12)

Anamnesis

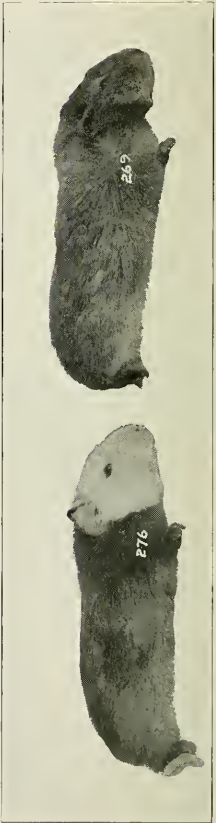
Sept. 16—Cheeks flushed.

Sept. 17—Patient vomiting, high fever, irrational, grinding teeth. Spasmodic twitching of hands, constipation, pain in lumbo-sacral region and posterior muscles of left leg. Headache and pain over left eye. Patient in attempting to stand fell over.

Sept. 19—Pupils dilated, facial and neck muscles normal. Well marked posterior and cervical adenopathy. Temperature 101°. Spasmodic twitching of pectoralis. Partial paralysis of lower left extremity.

Diagnosis: Poliomyelitis

Urine as submitted in sterile container September 27, 1921, proved positive to B. botulinus type A.



FIGS. 4 AND 5.—GUINEA PIGS 269 AND 276. PHOTOGRAPHS TAKEN FORTY-EIGHT HOURS AFTER TREATMENT

THE RELATION OF STRAINS 154, 269, AND 276 TO B. BOTULINUS TYPE A

Ten days after the urine and stool inoculations (154, 269, and 276) were made, the cultures were tested for toxicity by feeding .5 cc to healthy guinea pigs. The toxic effect of cultures 269 and 276 was first noted twenty-four hours after feeding. On the second morning the photographs shown in Figs. 4 and 5 were made, and within a few hours, forty-eight hours after the feeding of the toxin, the pigs were dead. The prostrate condition as observed in these animals is typical of botulinus intoxication in guinea pigs. Death in animals so affected generally follows in a few hours.

The lethal character of the cultures isolated from specimens 154, 269, and 276 to type A botulinus was demonstrated by injecting exposed guinea pigs with antitoxin. The results encountered in culture 154 are identical with those obtained from culture 276. Culture 269 produces a very mild extra-cellular toxin. On October 4, 1921, three guinea pigs were given .5 cc of broth culture 154 via the mouth. Simultaneously pig 76 was given subcutaneously 25 units of botulinus antitoxin type A, and pig 780 was given subcutaneously 25 units of

TABLE 1

G.P.	Toxin 10-4-21	Antitoxin 10-4-21	Results
76	154—.5 cc per oreum	25 units botulinus anti-toxin type A, subcutaneously	Remained healthy
780	154—.5 cc per oreum	25 units botulinus anti-toxin type B, subcutaneously	Dead 10-5-21
308	154—.5 cc per oreum	Control	Dead 10-5-21



FIG. 6.—GUINEA PIGS 76, 780, AND 308, SHOWING PROTECTIVE CHARACTER OF TYPE A ANTITOXIN. PHOTOGRAPH TAKEN TWENTY-FOUR HOURS AFTER TREATMENT

botulinus antitoxin type B. Pig 308 was left as a control. The treatment as administered and the result are presented in Table 1. In Fig. 6 is shown a photograph of the guinea pigs taken twenty-four hours after the treatment.

The immunologic relation of culture 154 to culture 276 was established by a similar procedure wherein the protective character of botulinus antitoxin type A was observed. The toxin and antitoxin were administered as indicated in Table 2. As shown in this same table, the pig which had been injected with botulinus antitoxin type A was the only one of the three which survived at the end of twenty-four hours. See Fig. 7.

TABLE 2

G.P.	Toxin 10-21-21	Antitoxin 10-21-21	Results
856	276—.5 cc per orem	25 units botulinus antitoxin type A, subcutaneously	Remained healthy
725	276—.5 cc per orem	25 units botulinus antitoxin type B, subcutaneously	Dead 10-22-21
124	276—.5 cc per orem	Control	Dead 10-22-21



FIG. 7.—GUINEA PIGS 856, 725, AND 124, SHOWING PROTECTIVE CHARACTER OF TYPE A ANTITOXIN. PHOTOGRAPH TAKEN TWENTY-FOUR HOURS AFTER TREATMENT

The toxogenic character of strain 276 for monkeys was observed by feeding the contents of one glucose agar shake culture to a healthy ringtail monkey (white-throated Sapajou or Capuchin, *Cebus hypoleucus*, No. 734). The culture was fed at 11 a.m., October 22, and the animal then placed in a separate cage and fed wholesome food. Profound toxic symptoms were observed at 7 a.m., October 24. At this time the monkey was able to stand erect for only a moment. An opossum-like attitude (Fig. 8) was assumed as the pendulous head



FIG. 8



FIG. 9



FIG. 10



FIG. 11

FIGS. 8-11.—SYMPTOMS OBSERVED IN MONKEY 734 FORTY-FIVE HOURS AFTER EXPOSURE

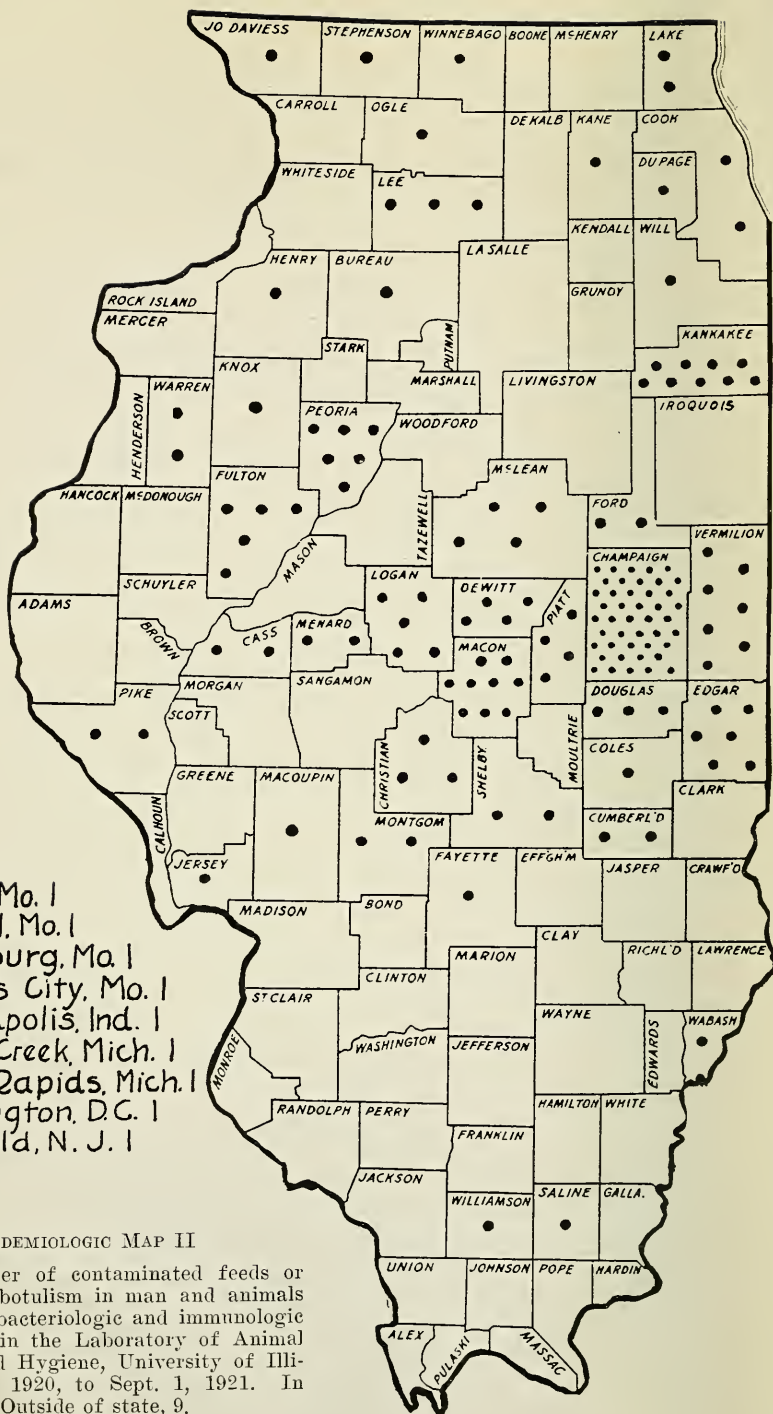
and neck (Figs. 9 and 10) flexed on the forearms. When placed in a standing position with the head erect, the animal fell over and assumed a lateral decumbent position (Fig. 11). The respirations were shallow and irregular. Following exertion, dyspnea and mouth-breathing were pronounced. Complete prostration and coma terminated in respiratory paralysis and death at 11 a.m., October 24.

SUMMARY

Approximately two hundred specimens of body excretions including feces and urine as well as throat swabs from some eighty cases of poliomyelitis are being examined in the Laboratory of Animal Pathology and Hygiene of the University of Illinois, for pathogenic anaerobes. One stool and five urines as submitted from widely separated spontaneous cases of this disease in the state of Illinois have proved positive to *B. botulinus* type A. The syndrome in each of the six cases was regarded as typical of poliomyelitis by the attending physician.

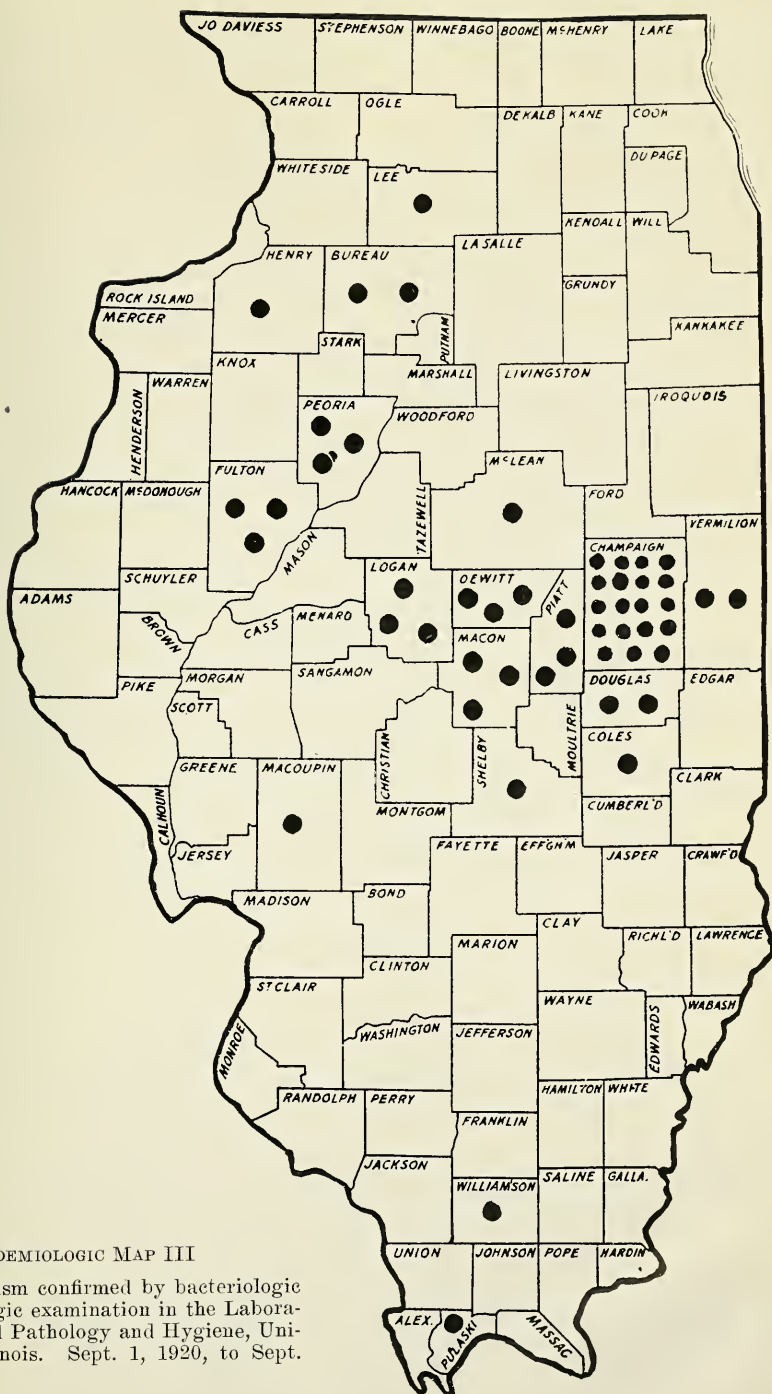
Since other possible factors have not been considered in the preliminary studies, the specific relation of *B. botulinus* type A to poliomyelitis-like diseases is only a matter of conjecture at this time. Furthermore, the number of cases of poliomyelitis in children included in this phase of the investigation is too small to permit the conclusion that *B. botulinus* is an etiologic factor. However, the possibility of a botulism transmission thru milk is experimentally established in animals, and if the human organism reacts similarly, milk may be a factor tho the possibility of transmission thru other foods merits consideration. The rôle of flies or other insects which might mechanically contaminate food, is not excluded in the preliminary studies. In view of these facts, as well as of the neurotropic character of botulinus toxin, it seems advisable not to overlook the possible relation which may exist between some cases of poliomyelitis in children and a specific botulinus intoxication, altho the occurrence of distinct and separate diseases in children, diagnosed as poliomyelitis, is not excluded in this connection.

The fact that *B. botulinus* type A has been encountered in the excreta of six convalescent poliomyelitis patients suggests the advisability of continuing the investigations until the saprophytic and toxogenic character of this microorganism in nature is definitely established with special reference to its presence in the excretions of normal children and of patients suffering from a paralytic syndrome.



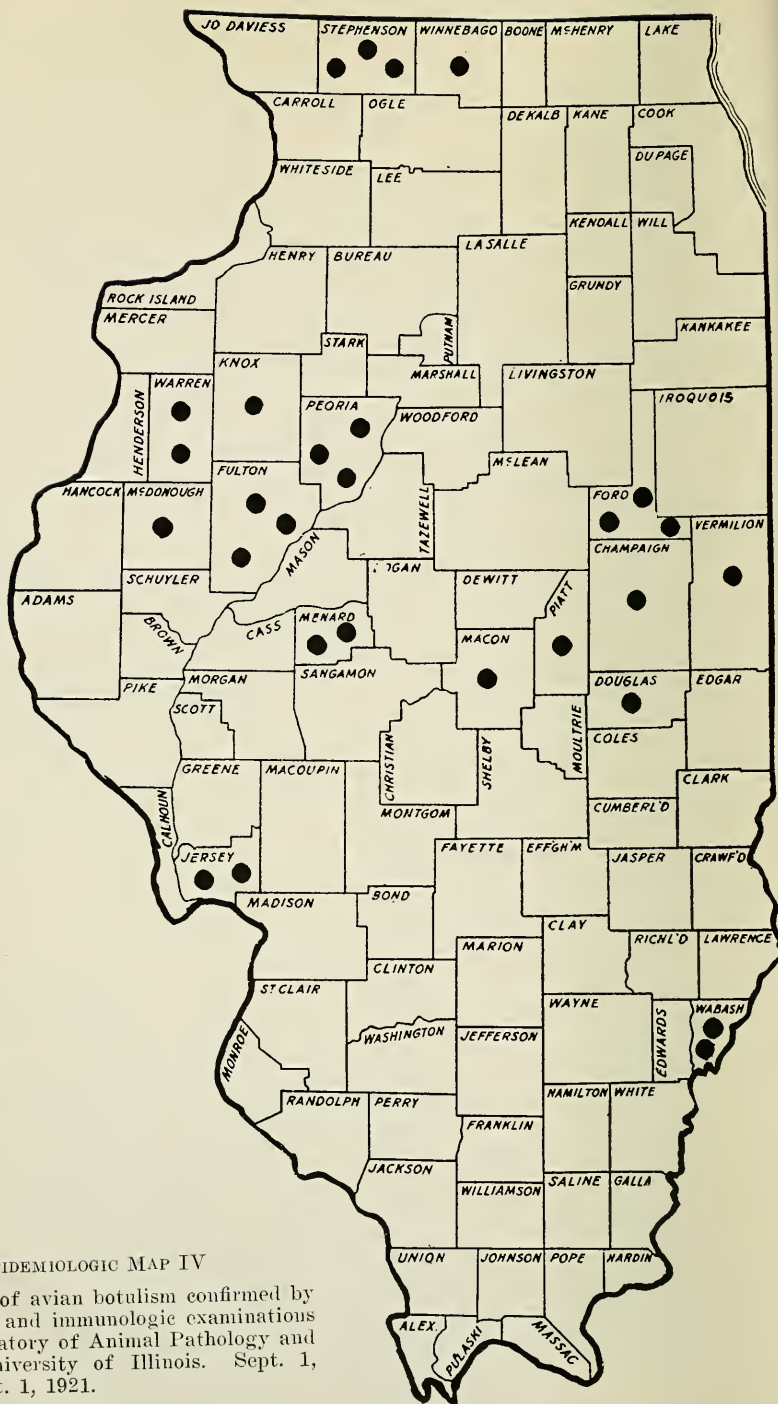
EPIDEMIOLOGIC MAP II

Total number of contaminated feeds or outbreaks of botulism in man and animals confirmed by bacteriologic and immunologic examinations in the Laboratory of Animal Pathology and Hygiene, University of Illinois, Sept. 1, 1920, to Sept. 1, 1921. In Illinois, 149. Outside of state, 9.



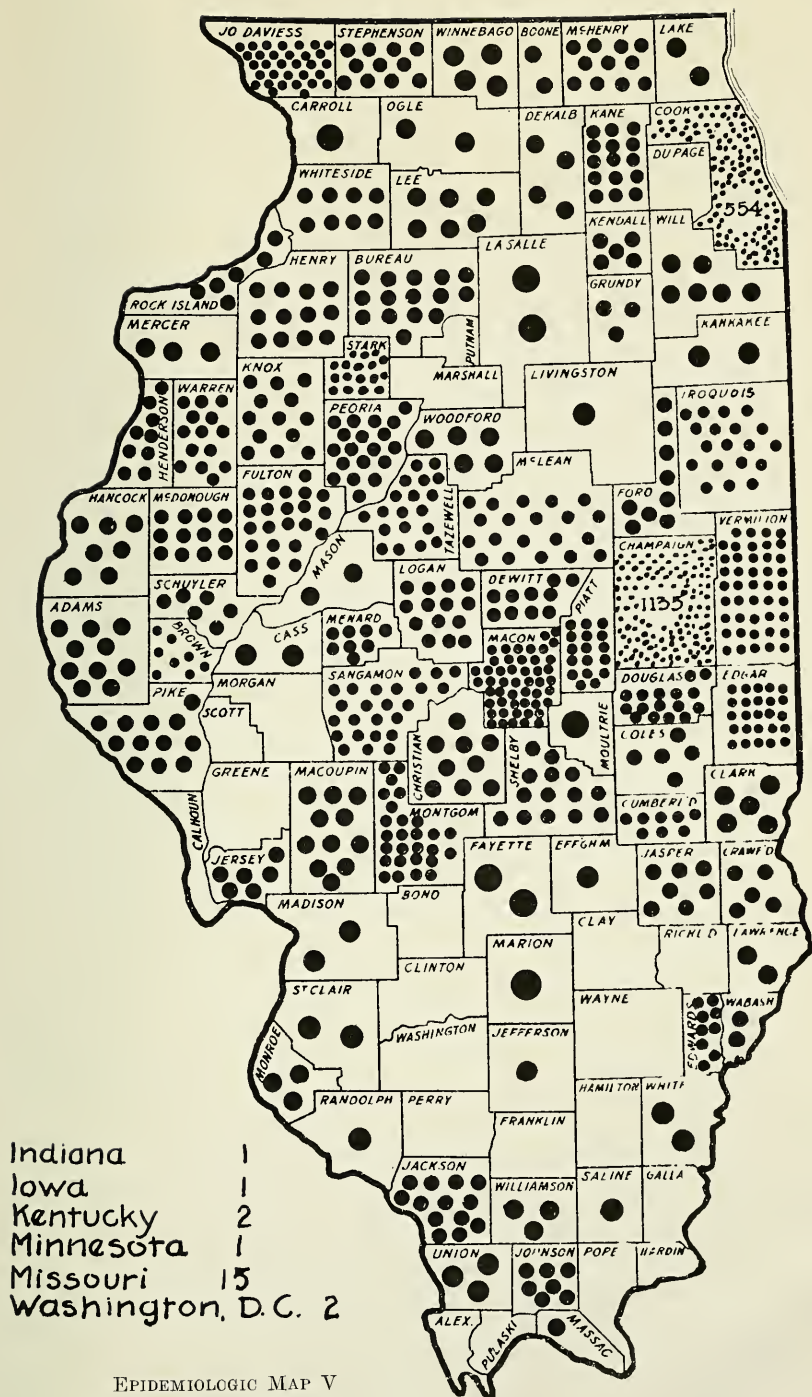
EPIDEMIOLOGIC MAP III

Swine botulism confirmed by bacteriologic and immunologic examination in the Laboratory of Animal Pathology and Hygiene, University of Illinois. Sept. 1, 1920, to Sept. 1, 1921.



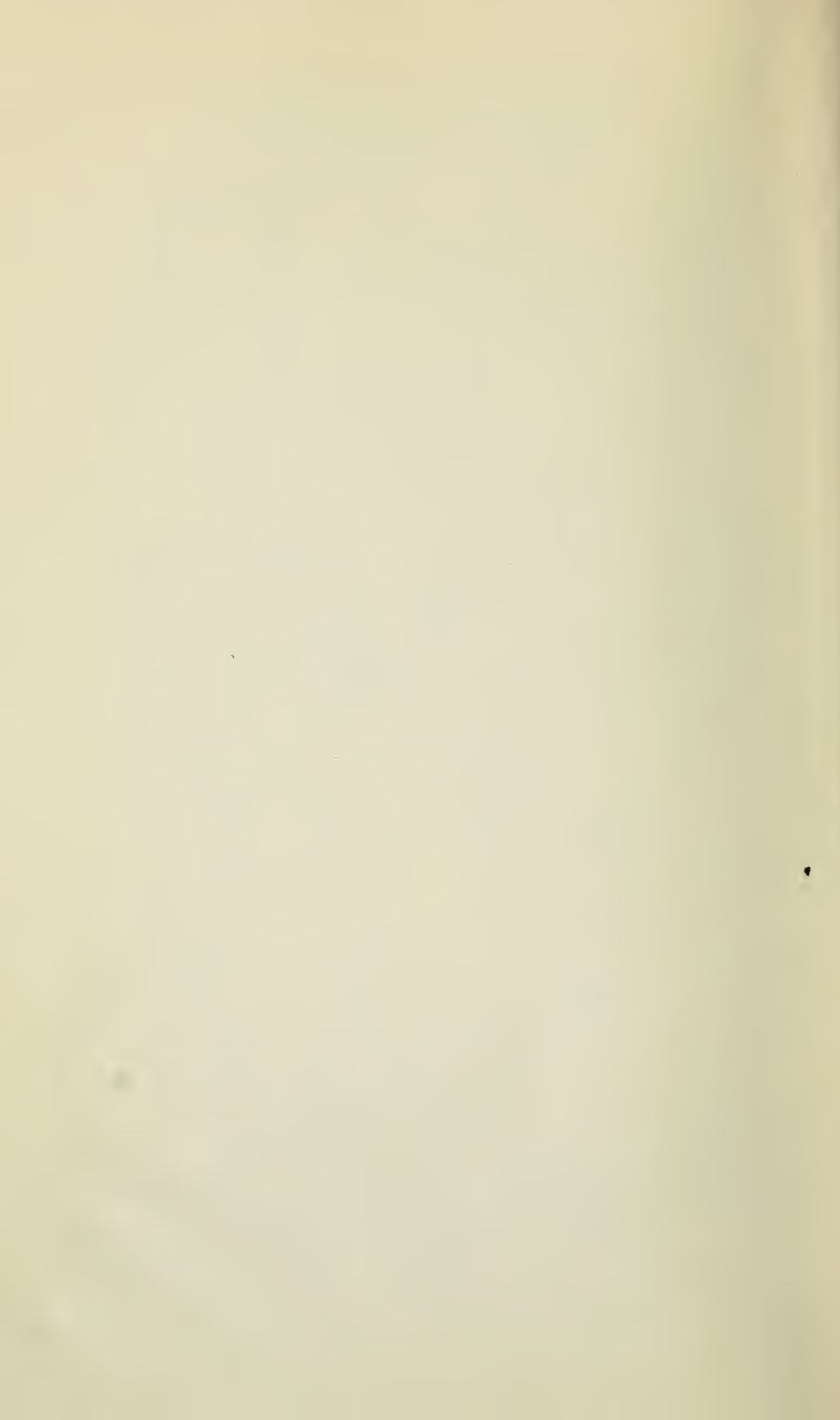
EPIDEMIOLOGIC MAP IV

Outbreaks of avian botulism confirmed by bacteriologic and immunologic examinations in the Laboratory of Animal Pathology and Hygiene, University of Illinois. Sept. 1, 1920, to Sept. 1, 1921.



EPIDEMIOLOGIC MAP V

2505 specimens submitted to the Laboratory of Animal Pathology and Hygiene, University of Illinois, for bacteriologic, pathologic, and serologic diagnosis. Sept. 1, 1920, to Sept. 1, 1921.



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